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Therapy

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13. ABSTRACT (Maximum 200 Words) Background: Combination of the cytotoxic viral thymidine kinase (tk) and the prodrug, acyclovir (ACV) has been reported to inhibit the growth of the C4-2 tumor, a subline of LNCaP. However, it remains unsolved to non-invasively detect the in vivo distribution, expression and persistence of the toxic gene as well as to evaluate the therapeutic effect. In this project, we will develop a nuclear gene imaging approach to assist the cytotoxic gene therapy study for prostate cancer. Objective/Hypothesis: The distribution, expression, and persistence of the prostate specific Ad-PSA-tk in the C4-2 tumor xenograft model will be non-invasively and repeatedly determined in vivo by tracing the radiolabeled TK substrates with a SPECT imaging modality. Specific Aim of the first year: To synthesize a radiolabeled TK substrate, 2'-Keoxy-2'-fluoro-5-{3-oxo[N,N-bis(2-mercaptoethyl)ethylenediaminato][Tc-99m]technetium(V)-1(E)-propenyl}uridine, for TK detection using a small animal gamma detector. Progress and outcome: In last report of 2003 which covers from September of 2002 to March of 2003, we reported our efforts to synthesize fragments A and B. In this report, we successfully linked the radiometal chelator with fluorothymidine. We will characterize the structure of the final tracer and test the pharmacokinetics and pharmacodynamics of the tracer in next research year. Also, the Adenoviral vectors with reporter genes of tk and luciferase were constructed. The luciferase gene expression in live mouse model was non-invasively imaged and the result was posted in 2003 Annual Meeting of ASGT (American Society of Gene Therapy).				
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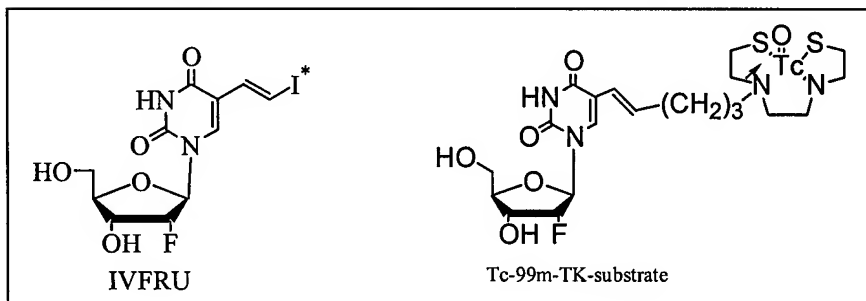
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Introduction

The objective of this project is to develop a noninvasive imaging assay using single photon emission computed tomography (SPECT) for assessment of gene therapeutic efficacy and diagnosis of metastasis of prostate cancer.

Currently, nuclear imaging technology has demonstrated the greatest potential to non-invasively image gene activity in animals and humans due to its high sensitivity. By replacing acyclovir (ACV) with a radioactive analogue, it is possible to non-invasively and repeatedly monitor the *in vivo* distribution of the transduced tk construct. It may assist in determining the optimal timing for ACV administration, confirming the cytotoxic sites, and assessing the therapeutic efficacy. Further refinement of this technology could also provide a non-invasive approach to identify any metastasis sites in a clinical setting.



In the original plan, we proposed to synthesize a novel thymidine kinase (TK) substrate, I-123 labeled 1-(2-deoxy-2-fluoro-β-D-ribofuranosyl)-

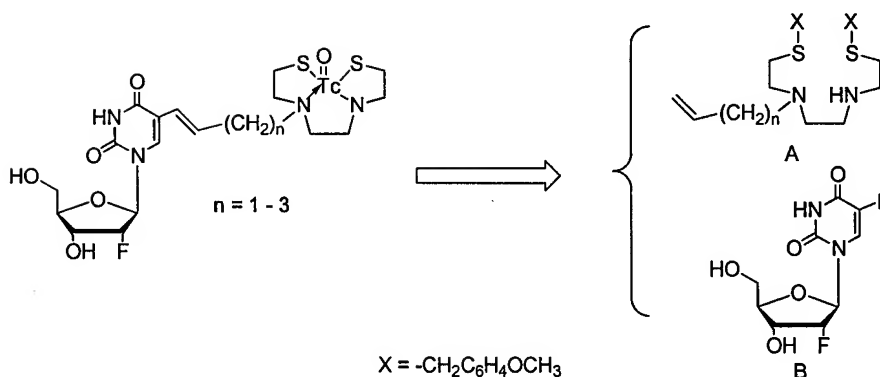
5(E)-(2-iodovinyl)uracil (IVFRU). However, the recent progress of Tc-99m chemistry of Tc-99m labeled radiopharmaceuticals, such as TRODAT, being able to penetrate lipid membrane raises our interest to synthesize a Tc-99m labeled TK substrate for gene imaging, because of the nearly optimal nuclear properties of Tc-99m, as well as its convenient and low cost production by means of commercial generator columns. As a result, we modified our plan by switching the target molecule, [I-123]IVFRU with 2'-Deoxy-2' fluoro-5-{3-oxo[N,N-bis(2-mercaptoethyl)ethylenediaminato]}[Tc-99m] technetium(V)-1(E)-propenyl}uridine.

In the biological experiment, we constructed a prostate specific adenovirus vector, Ad-PSA-TK. To test the target specificity of the PSA promoter, viral vector Ad-PSA-Luc was constructed and a charge coupled device (CCD) video camera was used to image noninvasively human prostate tumors and metastases in nude mice after injection of 2x10⁹ PFU of Ad-PSA-Luc virus via the tail vein.

Body

1. Chemistry

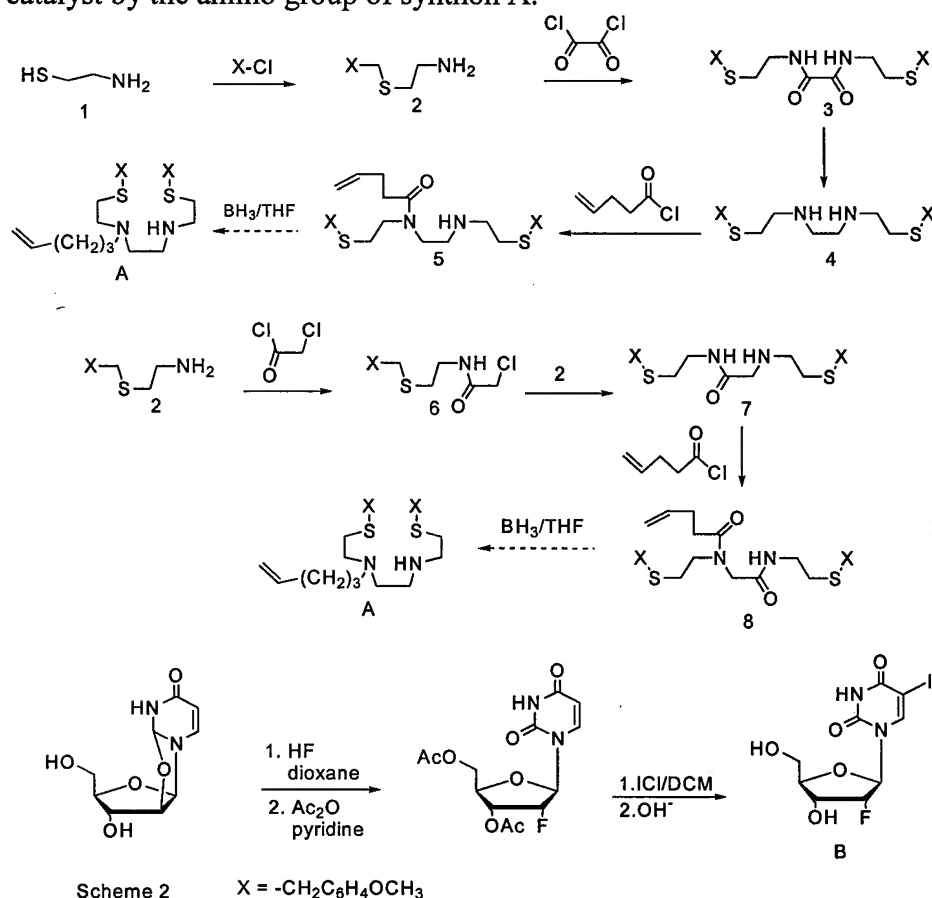
The target molecule will be convergently synthesized from compounds A and B.



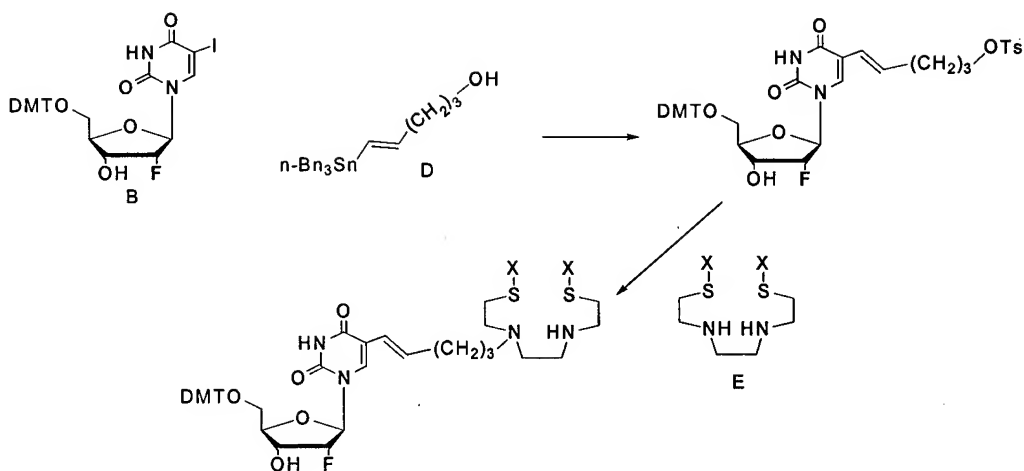
Scheme 1

Synthetic progress

In the 2003 report, the compound A and B were synthesized following the procedure depicted in Scheme 1. However, the proposed Heck coupling reaction of synthon A and B (Scheme 1) failed probably due to the deactivation of the palladium catalyst by the amino group of synthon A.

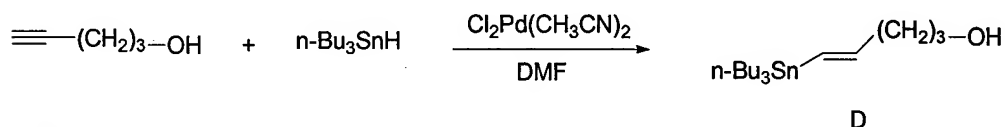


Scheme 2



Scheme 3 X = -CH₂C₆H₄OCH₃

As an alternative, the target molecule was synthesized by consecutive coupling reactions of three moieties (Scheme 3). The trans vinyl tin D was prepared by addition reaction of 5-hydroxyl pentyne with tributyltin hydride with a yield of 45% (Scheme 4).



Scheme 4

2. Biology

Viral Vector

Using pAdEasy1 systems, we constructed adenovirus vectors Ad-PSA-Luc and Ad-ACM-Luc, which can express firefly luciferase under the control of the 5837 bp long prostate-specific antigen promoter.

PSA specificity

Virus AdPSA-Luc or AdCMV-Luc was injected into mice with prostate cancer model via the tail vein. The image was performed on days 2, 4, 7, 9, 11. The CCD signals were quantified as relative light units per minute of acquisition time (RLU/min). On day 9, the outlines of lungs of the prostate mode mice injected with Ad-PSA-Luc were imaged distinctly. On day 11, mice were sacrificed and isolated organs were imaged. The signal in the whole lung displayed about 60 folds higher than in the whole liver. As a control, Ad-CMV-Luc containing the CMV promoter and luciferase gene was injected into nude mice with human prostate tumor models or via the tail vein. The strong liver signals (n=3) could be seen from 2 to 11 days after injection. The signals in the isolated livers from sacrificed mice on day 11 appeared about 600 folds higher than in the lungs. According to these results, AdPSA-Luc displayed about 30,000 folds higher specific expression in lungs than AdCMV-Luc.

Key Research Accomplishments:

Chemistry: The precursor of the Tc-99m labeled imaging tracer, 2'-Deoxy-2'fluoro-5-{3-oxo[N,N-bis(2-mercaptoethyl)ethylenediaminato][Tc-99m] technetium(V)-1(E)-propenyl}uridine, was successfully synthesized.

Biology: The preliminary imaging study demonstrated that the Ad-PSA-Luc virus shows significantly higher specificity towards prostate cancer than Ad-ACM-Luc.

Reportable Outcomes:**Conclusions:**

We will obtain the Tc-99m labeled TK substrate and evaluate its pharmacokinetics and pharmacodynamics as a reporter probe of TK gene expression.

Reference:

1. Detection of Metastases of Human Prostate Cancer Cells in Human Prostate Cancer Models with Noninvasive imaging, Hongwei Li, Gregory A. Helm, Jialing Hsieh, Leland Chung, Dongfeng Pan, *The 6th Annual Meeting of the American Society of Gene Therapy*, June 4-8, 2003, Washington, DC.